Correlates of pneumococcal virulence in the mouse

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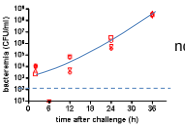
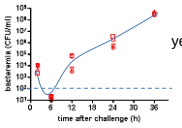
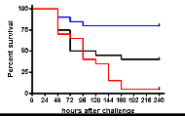
Disease or no disease: at the LD50



no



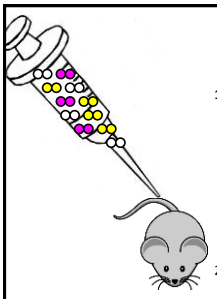
yes



Bacterial counts after challenge
- what happens to bacteria injected IV ?
- why do bacterial counts decrease ?
- how much do they decrease ?

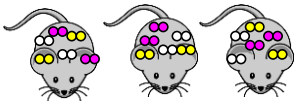
At the LD50 (lethal dose 50)
- what happens in mice **not** developing invasive infection ?
- what happens in mice developing invasive infection ?

- *All data reported in this presentation are form obtained in an intravenous sepsis model in mice*

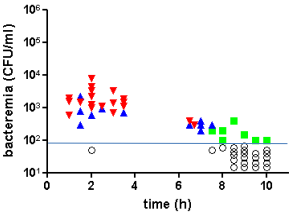


Challenge with one bacterium does not determine disease

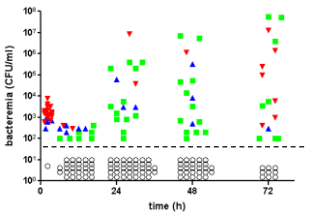
1. **Cooperative hypothesis**: Sepsis results from cooperative interactions between bacteria. Blood cultures of single mice should yield all pneumococcal variants.



2. **Single-organism hypothesis**: Sepsis results from an invasive event of a single bacterial cell. Blood culture from each mouse should yield a single pneumococcal variant.



Experimental design
- Intravenous (IV) infection with a mixture of three variants of the same pneumococcal strain
- *S. pneumoniae* TIGR4 (serotype 4) insertion of *ermB*, *aad9* or *adh6* in *zmpC*
- Dose 1 x 10^6 CFU/mouse in outbred CD1 mice (3x10^5 CFU each strain)



Prevalence of disease

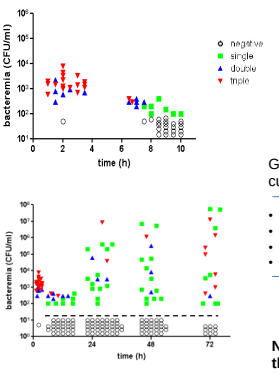
	infected	total	%
24h	13	36	36
48h	10	24	42
72h	7	12	58

Single variant infections

	Erm	Km	Spe
24h	5	2	1
48h	2	3	2
72h	2	3	2

Prevalence of variants in any infection

	Em	Km	Spe
24h	9	7	4
48h	4	6	4
72h	4	5	4



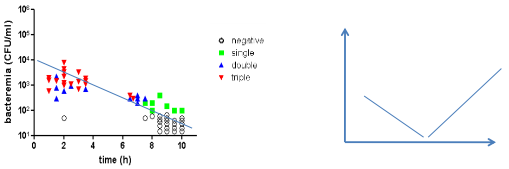
Is there evidence for within-host selection for fitness ?

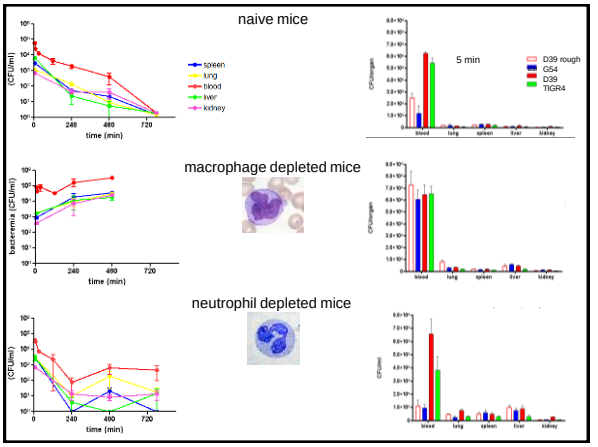
Genome sequence of 6 monoclonal blood cultures yielded:

- 2 clones; no mutation in 100% of population
- 2 clones; SNP in intergenic region in 100%
- 1 clone; silent SNP in murF 100%
- 1 clone; conservative SNP in hypot. Prot. 100%

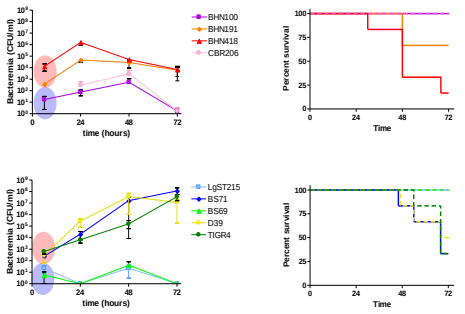
No within-host selection for fitness – the disease causing clone is selected by chance

What determines the bottleneck ?

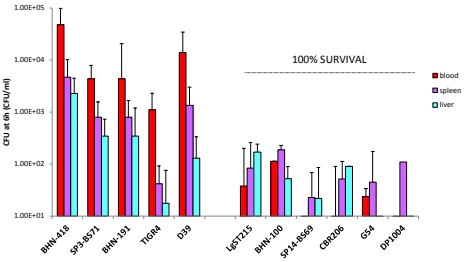




Can disease outcome be predicted from events in the first hours ?

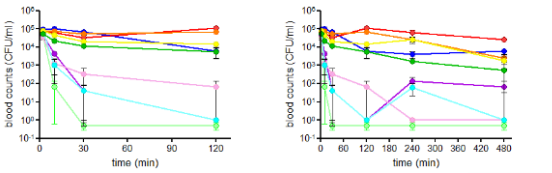


Bacterial counts in which organ correlate to outcome ?



- Also larger group of strains split into two groups
- Groups mainly differ for blood counts, while early organ counts are similar

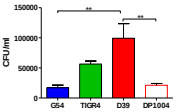
Can disease outcome be predicted from events in the first minutes ?



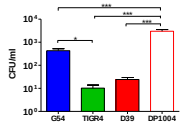
Non-virulent strains are cleared in few minutes after challenge, while virulent strains are more resistant

Can *in vitro* data predict virulence ?

Blood counts at 5 minutes

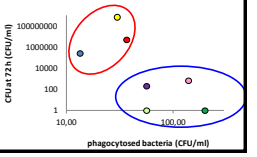


Phagocytosis by primary spleen macrophages

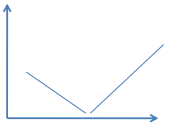


It is possible to "predict" virulence of pneumococci by *in vitro* test

(in sepsis model)
(most pneumococci – type 3 does not fit)



Virulence and disease is more than phagocytosis



Data for IV sepsis model	-	*carriage or pneumonia models*
Data for certain strains	-	*type III behaves differently*
Data on initial phases only	-	*neutrophils and "growth" in blood excluded*
Data focussed on spleen	-	*clearance mechanisms in liver excluded*
Data on non-immune mice	-	*humans have pre-existing antibodies*

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Pneumopath

PNEUMOPATH – WP3

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